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ADENOVIRUS INFECTION (AI) AND DISEASE (AD) IN PEDIATRIC MATCHED UNRELATED DONOR BONE MARROW TRANSPLANT (BMT) PATIENTS RECEIVING EITHER ANTI-THYMOCYTE GLOBULIN (ATG) OR CAMPATH DURING CONDITIONING

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Recent reports have shown an increased incidence of AI in patients treated with Campath. The incidence of AI/AD was compared in 112 patients at our institution who underwent allogeneic BMT receiving either ATG or Campath during conditioning. AI was defined as isolation of adenovirus in culture or detection by PCR from a single site. AD was defined as isolation/detection in blood or end organ involvement. Two patients with AI were excluded for receiving both medications. AI was present in 25% overall (27/110), AD was seen in 1.8% (2/110). Of those receiving ATG (72/110), AI rate was 24% (17/72) and AD was in 1.4% (1/72); for those receiving Campath (43/112) AI rate was 28% (13/43) and AD was in 2.3% (1/43) (p=0.69). Mortality for the entire group was 30% (34/112). Two of the 27 patients with AI died of AD. One patient received Campath and had grade IV graft versus host disease (GVH). The second patient received ATG and had no GVH. These findings suggest no difference in adenovirus associated infection and disease rates among those receiving conditioning containing either ATG or Campath. Several risk factors have been identified for AD in the BMT patient including prolonged CD4 suppression, grade II-IV GVH and viral isolation from multiple sites. We plan to further delineate risk factors by measuring viral load by real time PCR and monitoring adenovirus specific immune response by Elispot.

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DETECTION OF PEPTIDE-SPECIFIC ALLOREACTIVE T CELLS USING HLA CLASS I TETRAMERS

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After stem cell or solid organ transplantation alloreactive T cells are stimulated and form a vigorous response to allogeneic HLA molecules. In the absence of aggressive immunosuppression strategies these cells cause graft versus host disease and graft rejection. We sought to obtain evidence that the magnitude of the T cell alloresponse results from the numerous potential antigenic targets created by the diverse range of peptides presented by allogeneic HLA molecules. HLA tetrameric complexes were used to identify the ligands recognised by alloreactive T cells. A panel of A*0201/peptide tetrameric complexes was generated representing abundant self-peptides known to bind endogenously to A*0201. Alloreactive CD8+ T cell lines specific for A*0201 were stimulated in vitro and screened for tetramer binding by flow cytometry to identify and quantify cells that recognise each HLA/peptide alloantigen. For all HLA/peptide combinations tested a small population of tetramer binding CD8+ T cells was found. Tetramer-binding T cells exhibited exquisite specificity for the peptide bound by allogeneic A*0201 and peptide specific alloreactive T cells could be induced by both disparate and very limited HLA mismatches. These alloreactive T cells were shown to originate from the memory T cell population indicating that they represent cross reactive T cells previously stimulated by antigenic peptides from foreign pathogens presented by self MHC molecules. TNF α expression along with enhanced expression of perforin and IFN γ was detected in tetramer binding cells demonstrating that the peptide alloreactive T cells are functional. Our results provide evidence to support the theory that the vigorous alloreactive T cell response is caused by the summation of numerous responses to each of the peptides bound by the allogeneic HLA molecules and show that tetramers can be used to detect functional alloreactive T cells. The panel of tetramers is currently being used to screen for alloreactive T cells in HLA mismatched transplant patients.

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SELECTIVELY DEPLETING HOST-REACTIVE T CELLS FROM PERIPHERAL BLOOD STEM CELL ALLOGRAFTS - PRELIMINARY RESULTS OF A FEASIBILITY STUDY

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Ex vivo selective depletion (SD) is a strategy to prevent graft-versus-host-disease (GVHD), in which host-reactive donor lymphocytes are selectively eliminated from a peripheral blood stem cell (PBSC) allograft while conserving useful donor immune function. We initiated a clinical study to assess the feasibility of this approach in the setting of nonmyeloablative stem cell transplants (NST) in older patients with hematologic malignancies. Three patients (median age 66 years) with advanced myelodysplastic syndrome (2 RAEB, 1 RAEB-AML) received a low intensity preparative regimen consisting of cyclophosphamide and fludarabine, followed by a "selectively-depleted" PBSC allograft from an HLA-identical sibling donor. To obtain such a graft, a positive/negative selection (Isolex 300i) was performed on G-CSF-mobilized donor peripheral blood to generate a stem cell-rich product containing a CD34+ cell dose of $\geq 3x10^6$/kg and a CD3+ cell dose of $5x10^4$/kg. The remaining fraction was then co-cultured with irradiated lymphocytes obtained from the patient before transplant by apheresis. After 72 hours an anti-CD25 immunotoxin, RFT5-SMPT-dgA, was added to remove alloreacting cells. The washed T cell product (CD3+ cell dose of 1-2 x 10^8/kg) was infused following the preparative regimen, together with the stem cell-rich product. All patients received post-transplant immunosuppression with cyclosporine for a minimum of 30 days, followed by dose reduction depending on the degree of donor lymphocyte chimerism. SD lymphocytes were successfully generated for all patients, meeting release criteria for cell numbers, viability, and sterility and were infused safely without any untoward effects. All patients achieved 100% donor T cell engraftment by day 30, comparable to that seen with unmanipulated NST. Rapidly-resolving grades I and II skin-GVHD occurred in two patients, and no patient died of transplant-related causes. This is in contrast to a 35% TRM seen in a previous cohort of older patients receiving the same protocol without SD. Two survive 43-365 days post-transplant, one patient (with AML at time of transplant) died day 200 of progressive leukemia. These preliminary results are promising and demonstrate the ability of SD lymphocytes to engraft.

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ANTI-LEUKEMIC ACTIVITY OF CHRONIC GRAFT-VERSUS-HOST DISEASE FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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The aim of this study was to evaluate the outcomes for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) in remission treated with allogeneic bone marrow transplantation (BMT). Twenty-three adults were entered onto this study. The 2-year probabilities of relapse and disease-free survival (DFS) were 39.4% and 43.5%, respectively. The presence of chronic graft-versus-host disease (GVHD) was found to be an independent predictive factor affecting lower relapse and DFS. To monitor the BCR-ABL transcript, we also analyzed 48 bone marrow samples of 8 patients using real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). The kinetics of the normalized amount of BCR-ABL transcript (BCR-ABL/ABL) were well correlated with their clinical courses. In 6 patients with continuous remission after BMT, a rapid decrease in BCR-ABL amount to the PCR negative status after the development of chronic GVHD was observed. Meanwhile, routine bone marrow